Pharmacogenomics and Personalized Medicine

Dovepress

open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Influence of SLCO1B1 521T>C, UGT2B7 802C>T and IMPDH1 −106G>A Genetic Polymorphisms on Mycophenolic Acid Levels and Adverse Reactions in Chinese Autoimmune Disease Patients

Pharmacogenomics and Personalized Medicine downloaded from https://www.dovepress.com/
For personal use only.

Qing Shu[1,*]
Qingqing Fan[1,*]
Bingzhu Hua[2]
Hang Liu[1]
Shiying Wang[2]
Yunxing Liu[1]
Yao Yao[1]
Han Xie[1]
Weihong Ge[1]

[1]Department of Pharmacy, Nanjing Drum Tower Hospital, Nanjing, 210008, People's Republic of China; [2]Department of Rheumatology and Immunology, Nanjing Drum Tower Hospital, Nanjing, 210008, People's Republic of China

*These authors contributed equally to this work

Introduction: Mycophenolate mofetil (MMF), a new type of immunosuppressant, has emerged as a frontline agent for treating autoimmune diseases. Mycophenolic acid (MPA) is an active metabolite of MMF. MPA exposure varies greatly among individuals, which may lead to adverse drug reactions such as gastrointestinal side effects, infection, and leukopenia. Genetic factors play an important role in the variation of MPA levels and its side effects. Although many published studies have focused on MMF use in patients after organ transplant, studies that examine the use of MMF in patients with autoimmune diseases are still lacking.

Methods: This study will not only explore the genetic factors affecting MPA levels and adverse reactions but also investigate the relationships between UGT1A9 −118(dT)9/10, UGT1A9 - 1818T>C, UGT2B7 802C>T, SLCO1B1 521T>C, SLCO1B3 334T>G, IMPDH1 −106G>A and MPA trough concentration (MPA C_0), along with adverse reactions among Chinese patients with autoimmune diseases. A total of 120 patients with autoimmune diseases were recruited. The MPA trough concentration was detected using the enzyme multiplied immunoassay technique (EMIT). Genotyping was performed using a real-time polymerase chain reaction (PCR) system and validated allelic discrimination assays. Clinical data were collected for the determination of side effects.

Results: SLCO1B1 521T>C demonstrated a significant association with MPA C_0/d (p=0.003), in which patients with the CC type showed a higher MPA C_0/d than patients with the TT type (p=0.001) or the CT type (p=0.000). No significant differences were found in MPA C_0/d among the other SNPs. IMPDH1 −106G>A was found to be significantly related to infections (p=0.006). Subgroup analysis revealed that UGT2B7 802C>T was significantly related to Pneumocystis carinii pneumonia infection (p=0.036), while SLCO1B1 521T>C was associated with anemia (p=0.029).

Conclusion: For Chinese autoimmune disease patients, SLCO1B1 521T>C was correlated with MPA C_0/d and anemia. IMPDH1 −106G>A was significantly related to infections. UGT2B7 802C>T was significantly related to Pneumocystis carinii pneumonia infection.

Keywords: mycophenolic acid, gene polymorphisms, adverse drug reactions, infections, anemia, autoimmune diseases

Introduction

Mycophenolate mofetil (MMF) is a new type of immunosuppressant with the active component metabolite mycophenolic acid (MPA). MPA is a selective hypoxanthine single-nucleotide dehydrogenase inhibitor. It blocks the synthesis of guanine

Correspondence: Weihong Ge
Department of Pharmacy, Nanjing Drum Tower Hospital, Nanjing, 210008, People's Republic of China
Email 6221230@sina.com

Received: 5 February 2021
Accepted: 20 May 2021
Published: 21 June 2021

© 2021 Shu et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v3.0) License (http://creativecommons.org/licenses/by-nc/3.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

nucleotides by inhibiting the activity of inosine-5′-monophosphate dehydrogenase (IMPDH) in the purine synthesis pathway, thus inhibiting DNA synthesis. MMF has been widely used as anti-rejection therapy in patients with organ transplant. Because of the efficacy and safety of MMF in organ transplant recipients, increasing emerging evidence suggests the successfulness of utilizing MMF in treating patients with systemic lupus erythematosus, Sjogren's syndrome, and other autoimmune diseases.[1–4]

MMF has been widely used because it is well tolerated and safer than other immunosuppressive agents and has fewer toxic side effects. Many studies have confirmed a positive correlation between MPA exposure and efficacy. However, MPA exposure has wide interindividual differences among the same ethnic group.[5–8] MPA exposure exhibits large interpatient variation among individuals by approximately 7- to 10-fold, resulting in different degrees of immunosuppression.[7] There was broad interindividual variability in clinical efficacy or adverse drug reactions (ADRs) among different individuals under a fixed dose. While insufficient immunosuppression will not achieve a satisfactory treatment effect, excessive immunosuppression might lead to ADRs, such as gastrointestinal side effects, infections, leukopenia, anemia, and low platelet count.[9,10]

Additionally, there is a wide ethnic variation in the pharmacokinetics of MPA. Compared to Western patients, Asian patients have higher MPA exposure; thus, more adverse events occur in Asian patients when taking fixed-dose MMF. The optimal MMF dose of Asian patients is 20–46% lower than that of Caucasians or African Americans.[11,12] Many available research studies are examining the use of MMF in patients with organ transplant. The dosage of MMF in treating patients with autoimmune diseases is much lower than that in patients with organ transplant; thus, more analytic reviews focusing on MMF in patients with autoimmune diseases are needed. It is crucial to gather more evidence to support pharmacokinetic variation to achieve the essential plasma MPA concentration and to avoid potential adverse reactions.

Gene polymorphisms of transporters and enzymes are the main factors that affect individual variations in drug exposure. MMF is rapidly absorbed in the gastrointestinal tract after oral administration and transforms to MPA by esterase. MPA undergoes enterohepatic cycling by organic anion transporting polypeptide (OATP, encoded by SLCO) and is metabolized into 7-O-glucoside (mycophenolic acid glucuronide, MPAG) and acyl-glucuronide (mycophenolic acid acyl-glucuronide, AcMPAG) through uridine 5′-diphospho-glucuronosyltransferase (UGT). AcMPAG, a minor metabolite produced by UGT2B7, is highly reactive and thought to be related to potential adverse events.[13–15] Gene polymorphisms involved in MPA disposition may also contribute to the interindividual variability of MPA pharmacokinetics. The isoforms that are involved in the distribution, metabolism, and targeting of MPA include UGT1A9, UGT2B7, SLCO1B1, SLCO1B3, and IMPDH.[16]

Some studies have indicated that UGT1A9-118(dT) $_{9/10}$ affects the plasma levels of MPA and MPAG,[17,18] while others have come to the opposite conclusion.[17–19] Many studies suggest significant differences in the mutation frequencies of single-nucleotide polymorphisms (SNPs), such as the UGT1A9 −1818T>C, UGT2B7 802C>T, and IMPDH1 −106G>A genotypes, among Asian and European populations. These three SNPs are common genetic variations in the Chinese population. Some gene polymorphisms, such as IMPDH1 −106G>A, are directly related to adverse reactions.[20,21] It is clinically significant to investigate the effects of these SNPs on MPA pharmacokinetics and their adverse effects among Chinese patients.

Based on previous studies, this study aimed to explore the genetic factors affecting MPA levels and adverse reactions. This study investigated the relationships between the specific SNPs that are associated with the disposition of MMF in Chinese autoimmune disease patients and the MPA level, along with the adverse reactions. SNPs with large differences in frequency between Chinese and Western populations were also investigated.

Materials and Methods

Subjects

This study was approved by the Ethics Committee of Drum Tower Hospital (2019-039-01). And this study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all the participants. Patients with autoimmune diseases treated with mycophenolate mofetil were recruited from April 2018 to January 2019. Eligibility criteria included (1) diagnosis of autoimmune disease; (2) MMF treatment for at least one week; and (3) improvement after MMF treatment.

The exclusion criteria were as follows: (1) patients with other serious complications, such as severe infection

Table 1 PCR Primer Sequences

SNPs	Forward Primer F	Reverse Primer R
rs3832043	5′ ATTTAGGAGGTTAGGAGGTCAGTG 3’	5′ GGGAATCTAAGCTCCTATGATACAG 3’
rs13418420	5′ AGAAGCCTTACCAATAACAGAAACA 3’	5′ TCATGAATGGGCACTGAAACTA 3’
rs7439366	5′ GATGTAGCTTAACCTCACAATTCTC 3’	5′ CATTTCCCATCTTTCTTTCAGTGTA 3’
rs4149056	5′ GGCTTTGCTCTTCCTTCATCT 3’	5′ ACAAAGGGAAAGTGATCATACAATT 3’
rs4149117	5′ CTCATATAGCCAAATTACCCAAGTG 3’	5′ GGATAATCAAAATCTTACAGGCAAA 3’
rs2278294	5′ GCTCTGACCACACTTCCCTTCT 3’	5′ GATGAAGCCCTGTTCAAACTTCT 3’

or malignant tumors; and (2) combination use of the following drugs: cyclosporine, tacrolimus, thalidomide, metal-containing drugs, cholestyramine, probenecid, and iron-rich foods or drugs that can affect the absorption and metabolism of MMF.

The following dosing regimen was used: the MMF dosage range was 0.5–2 g/day, and the physician selected the appropriate dosage according to the patient's condition. Other drugs based on the patient's condition can be combined with glucocorticoids or hydroxychloroquine (HCQ). If adverse reactions occurred, after consultation with the physician, MMF was withdrawn or reduced in dosage if necessary.

Sample Collection and Determination of MPA

On the day of the patient's treatment, 2 mL venous blood was collected in the EDTA anticoagulation tube, 1 mL of the blood was sampled, and centrifuged at 4°C and 50g for 5 min, the supernatant was kept in −80 °C for storage. Enzyme multiplied immunoassay technique (EMIT) was used for detection of steady-state plasma concentration of MPA (MPA C_0), refer to the method provided in the kit (6R91945092, Siemens Healthcare diagnostics Inc., USA).

Genotyping

DNA was extracted from venous blood by using genome DNA extraction kit (B518253, Sangon Biotech, Shanghai). Genotypes were assessed for UGT1A9 −118(dT)9/10 (rs3832043), UGT1A9 −1818T>C (rs13418420), UGT2B7 802C>T (rs7439366), SLCO1B1 521T>C (rs4149056), SLCO1B3 334T>G (rs4149117) and IMPDH1 −106G>A (rs2278294) by using a real-time Polymerase chain reactions (PCRs) system (Applied Biosystems, USA).

Briefly, 1 ng of genomic DNA was mixed with each assay and PCR universal master mix (Sangon Biotech, Shanghai) in a total volume of 20 μL. Thermal cycler parameters included 25 cycles of denaturation at 96°C for 10s, annealing at 50°C for 5s, and extension at 60°C for 4min. The PCR primer sequences is shown in the Table 1.

Data Collection and Criteria for Adverse Reactions

The patients' clinical data were recorded, including (1) demographic information, such as sex, age, and diagnosis; (2) MMF daily dose and the course of treatment; (3) combined medications, ie, glucocorticoids, hydroxychloroquine and proton pump inhibitors; (4) laboratory tests related to adverse reactions of the hematological system, ie, white blood cell count (WBC), hemoglobin content (Hb), platelet count (PLT); and (5) laboratory tests related to infection, ie, routine urinalysis, including red blood cell count (RBC), WBC count, bacterial examination, cytomegalovirus DNA, Epstein-Barr (EB) virus DNA, fungal glucan (G test), aspergillus test (GM test), the results of sputum culture and drug sensitivity tests. All patients were followed up. MMF-related adverse drug reactions were recorded during the treatment.

The criteria for adverse reactions were as follows:

1. Gastrointestinal side effects: diarrhea, abdominal pain, nausea, and vomiting occurred due to unknown reasons during medication. Patients without fever and inflammatory diseases became better after the MMF was reduced or discontinued. There was no inducement of the above symptoms, excluding other pathogenic factors.
2. Infection: The occurrence and type of infection were identified by the infection history, infection-related symptoms, laboratory examination, and anti-infectious therapy during hospitalization and follow-up visits after consultation. The types of infection mainly included bacterial infection, fungal infection and viral infection. It was considered

infection when patients presented with clinical manifestations of infection, laboratory tests showed evidence of infection, and anti-infective treatment was effective.

3. Adverse reactions of the hematological system: White blood cell count, hemoglobin level, and platelet count before medication were recorded, and the aforementioned conditions were excluded if they were caused by other diseases. Leukopenia: WBC < 4 × 109/L; anemia: Hb < 110 g/L; thrombocytopenia: platelet count < 100 × 109/L; patients improved after reduction or discontinuation.

Data Analysis

Statistical analysis was performed using SPSS Statistics 22.0 software. Continuous variables are presented as the mean ± SD or median (lower quartile, upper quartile) according to the distribution characteristics. Categorical variables are expressed as percentages. Hardy-Weinberg equilibrium (HWE) test: The χ^2 test was used to test the distribution frequencies of genotypes. It was considered that the distribution frequency of genotype conformed to the HWE if $p > 0.001$, indicating that the included patients were representative of the population. The Kruskal–Wallis test was used to determine associations between the genotype and MPA C_0. The χ^2 test (R×C table) was used to evaluate the effect of genotype on adverse reactions. The difference was considered to be statistically significant when $p<0.05$.

Results

Patient Characteristics and Genotype Distribution

A total of 120 patients were included in the present study. Eighty-one cases (67.5%) were diagnosed with SLE, 21 cases (17.5%) with SS, and 18 cases (15.0%) with other autoimmune diseases, mainly including vasculitis, myositis/dermatomyositis and scleroderma. The average daily dose of MMF was 1.22 ± 0.48 g. The demographic characteristics are described in Table 2.

Table 2 Demographic Characteristics

Characteristics	
Sex (Female, %)	104 (86.7%)
Age, years	36.61±15.42
Type of autoimmune disease	
Systemic Lupus Erythematosus (SLE)	81 (67.5%)
Sjogren's Syndrome (SS)	21 (17.5%)
Other autoimmune diseases	18 (15.0%)
MMF Dose, g/d	1.22±0.48
Course of treatment, month	14±11

The genotype distributions of the SNPs are shown in Table 3. UGT1A9 $-118(dT)_{9/10}$ (rs3832043) genotyping was performed as follows: 9T: –; 9T10T: -T; 10T: TT. The HWE test was performed on the six SNPs investigated in this study. All the genotype distributions conformed with HWE ($p>0.001$) without UGT1A9 $-118(dT)_{9/10}$ (rs3832043) and SLCO1B3 334T>G (rs4149117). Due to the sex difference in the occurrence of autoimmune diseases, the number of female patients was significantly greater than that of male patients, which may be the reason for this result.

The Influence of the SNPs on MPA C_0/d

A total of 115 patients who met all the criteria were included. Five patients were excluded due to unqualified MPA C_0 results that exceeded the detection limit. In this study, the daily dose of MMF was 0.5–2 g, the median MPA C_0 was 1.54 (0.73, 3.29) μg/mL, the lowest was 0.02 μg/mL, and the highest was 15.12 μg/mL. The coefficient of variation (CV) of MPA C_0 in 117 patients was 104.0%,

Table 3 Genotype Distribution of the SNPs

SNPs		Major/ Minor Allele	Major Allele Homozygotes, n (%)	Heterozygote, n (%)	Minor Allele Homozygotes, n (%)	HWE p
UGT1A9 −118(dT)9/10	rs3832043	-/T	9T (–) 4 (3.33%)	9T10T (-T) 103 (85.83%)	10T (TT) 13 (10.83%)	0.000
UGT1A9 −1818T>C	rs13418420	T/C	TT 33 (27.50%)	CT 64 (53.33%)	CC 23 (19.17%)	0.417
UGT2B7 802C>T	rs7439366	C/T	CC 55 (45.83%)	CT 46 (38.33%)	TT 15 (19.83%)	0.084
SLCO1B1 521T>C	rs4149056	T/C	TT 91 (75.83%)	CT 22 (18.33%)	CC 7 (5.83%)	0.002
SLCO1B3 334T>G	rs4149117	G/T	GG 63 (52.07%)	GT 4 (3.31%)	TT 54 (44.63%)	0.000
IMPDH1 −106G>A	rs2278294	C/T	CC 24 (20.00%)	TC 60 (50.00%)	TT 36 (30.00%)	0.912

which indicated that there were wide individual differences in MPA C_0. MPA levels were compared based on different genotypes of SNPs. Every SNP was grouped by genotype into homozygotes of major alleles, heterozygotes, and homozygotes of minor alleles. MPA C_0 was dose-normalized to MPA C_0/d, thereby eliminating the influence.

According to the results, SLCO1B1 521T>C demonstrated a significant association with MPA C_0/d (p=0.003). Patients with the CC type showed a higher level of MPA C_0/d than those with the TT type (p=0.001), the CT type (p=0.000) and the TT +CT type (p=0.001). Although the difference between the TT type and CT type was not significant (p=0.354), the MPA level in patients with the CC type was higher than that in other patients. A comparison of MPA C_0/d between the SLCO1B1 521T>C groups is shown in Figure 1. For UGT1A9 −118$(dT)_{9/10}$ (rs3832043), UGT1A9 −1818T>C (rs13418420), UGT2B7 802C>T (rs7439366), SLCO1B3 334T>G (rs4149117) and IMPDH1 −106G>A (rs2278294), no significant difference was found in MPA C_0/d among these different genotypes. The MPA C_0/d values of the different genotypes for each SNP are shown in Table 4.

SLCO1B1 521T>C is associated with MPA C_0/d (p=0.003). The MPA levels in patients with the CC type were higher than those in the general population. Patients with the CC type showed a higher MPA C_0/d than those with the TT type (p=0.001), the CT type (p=0.000) and the TT +CT type (p=0.001).

Influence of the SNPs on Adverse Reactions

Adverse reactions of MMF mainly include gastrointestinal adverse reactions, infection, and adverse reactions of the hematological system. Detailed information on adverse reactions is shown in Table 5.

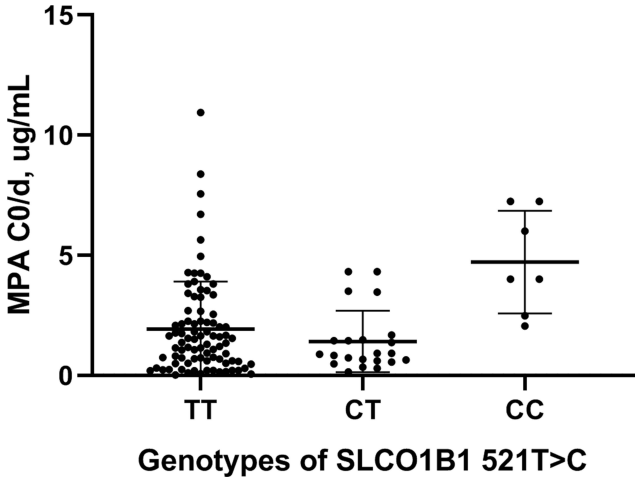

Figure 1 MPA C_0/d values of different genotypes for SLCO1B1 521T>C. MPA concentration (C0/d, ug/mL). TT CT CC: genotype of SLCO1B1 521 T>c.

The occurrence of adverse reactions was grouped to compare the distribution of genotypes among the ADR group and the normal group for each of the SNPs. The results showed that polymorphisms of UGT2B7 802C>T (rs7439366), SLCO1B1 521T>C (rs4149056) and IMPDH1 −106G>A (rs2278294) were correlated with infections and adverse reactions of the hematologic system, and no SNPs were associated with gastrointestinal adverse reactions.

The IMPDH1 −106G>A (rs2278294) gene polymorphism was significantly related to infections (p=0.006), and the distribution of the TC type in the infection group was much higher than that of the CC type (51.7% vs 16.7%, p=0.003) and the TT type (51.7% vs 30.6%, p=0.044). Subgroup analysis showed that UGT2B7 802C>T (rs7439366) was significantly related to Pneumocystis carinii pneumonia infection (p=0.036), and the distribution of the CT type in the Pneumocystis carinii pneumonia infection group was significantly higher than that of the CC type (15.2% vs 3.6%, p=0.092). For adverse reactions of the hematologic system, only anemia was affected by gene polymorphisms. The results showed that SLCO1B1 521T>C (rs4149056) was associated with anemia (p=0.029*), and the distribution of the CC type in the anemia group was statistically higher than that of the TT type (57.1% vs 17.6%, p=0.044). The correlations of SNPs and adverse reactions of different genotypes are shown in Table 6.

Discussion

This study evaluated the impact of UGT1A9 −118(dT)9/10, UGT1A9 −1818T>C, UGT2B7 802C>T, SLCO1B1 521T>C, SLCO1B3 334T>G and IMPDH1 −106G>A on plasma MPA levels and adverse reactions. Based on the limited available information, this study is the first conducted among Chinese patients, specifically focusing on patients with autoimmune diseases. The study results showed a significant association between SLCO1B1 521T>C and MPA C_0/d, and the MPA C_0/d values were significantly different among different genotypes. In addition, SLCO1B1 521T>C demonstrated positive MMF-induced anemia in Chinese autoimmune patients. IMPDH1-106G>A was correlated with MMF-induced infections, and UGT2B7 802C>T was correlated with Pneumocystis carinii pneumonia infection. This study was the first to verify these SNPs among Chinese patients with autoimmune diseases.

Table 4 MPA C_0/d of Different Genotypes for Each SNP

SNPs		MPA C_0/d, ug/mL			p
		Major Allele Homozygotes	Heterozygote	Minor Allele Homozygotes	
UGT1A9 −118(dT)9/10	rs3832043	9T 0.93 (0.11,-)	9T10T 1.45 (0.61,3.27)	10T 1.13 (0.54,1.96)	0.640
UGT1A9 −1818T>C	rs13418420	TT 1.16 (0.59,2.66)	CT 1.43 (0.65,2.26)	CC 1.45 (0.24,4.01)	0.888
UGT2B7 802C>T	rs7439366	CC 1.37 (0.66,2.16)	CT 1.80 (0.51,3.81)	TT 1.00 (0.58,2.12)	0.412
SLCO1B1 521T>C	rs4149056	TT 1.44 (0.56,2.58)	CT 0.90 (0.60,1.53)	CC 4.01 (2.48,7.24)	0.003*
SLCO1B3 334T>G	rs4149117	GG 1.42 (0.63,2.26)	GT 0.73 (0.70,-)	TT 1.45 (0.50,3.49)	0.953
IMPDH1 −106G>A	rs2278294	CC 1.43 (0.52,2.96)	TC 1.52 (0.56,3.30)	TT 1.29 (0.71,2.58)	0.943

Note: *p<0.05.

Table 5 Occurrence of Adverse Reactions

Types of ADRs	n, (%)
Gastrointestinal Side Effects	33 (28.2%)
Infections	45 (38.5%)
Bacterial Infection	38 (32.5%)
Viral Infection	7 (6%)
Pneumocystis Carinii Pneumonia Infection	9 (7.7%)
Adverse Reaction of Hematologic System	44 (37.6%)
Leukopenia	17 (14.5%)
Anemia	24 (20.5%)
Decreased Platelet Count	18 (15.4%)

The results of this study showed that the MPA C_0/d values were significantly different among different genotypes in SLCO1B1 521T>C. Among all, the MPA C_0/d value of the CC genotype group was significantly higher than those of the TT, CT, and TT + CT groups. Thus, the results of this study suggest that the MPA C_0/d for this genotype was higher than that of the general population. This result is different from previous studies. According to Ruiz et al[22] SLCO1B1 521T>C was not associated with dose-adjusted plasma MPA levels (C0/d) or adverse reactions in Caucasus transplantation patients. Similar results were obtained by Bouamar et al.[23] Although the frequencies of SLCO1B1 521T>C in Europe and East Asia are similar (0.1589 for Europe and 0.1254 for East Asia), the different racial groups were thought to be a contributing factor to the variability in MPA exposure.

OATP is mainly distributed in the liver and is involved in the uptake of MPA and MPAG. SLCO1B1 and SLCO1B3 gene polymorphisms may affect the metallization of MPA. Studies have shown that MPAG pharmacokinetics are affected by SLCO1B1 and SLCO1B3 polymorphisms.[24] Kagaya et al[25] indicated that patients with the SLCO1B3 334T>G TT genotype had a higher MPA $AUC_{0\text{-}12h}$. Miura[26] concluded that compared to patients with the TT genotype, the bile excretion of MPA in patients with the GG genotype was higher; thus, the MPA $AUC_{6\text{-}12h}$ was higher. Patients with the SLCO1B3 334T>G GG type showed higher levels of MPA uptake in hepatocytes and bile excretion. In this study, while SLCO1B3 334T>G did not affect MPA C_0/d, SLCO1B1 521T>C affected MPA C_0/d. The MPA C_0/d of CC patients was significantly higher than those of TT and CT patients, which is thought to be related to the decreased uptake and transportation of MPA by

Table 6 Correlation of SNPs and Adverse Reactions of Different Genotypes

Type of Adverse Reaction	SNPs	Without ADRs	With ADRs	p
Infection	rs2278294	CC 20 (83.3%) TC 29 (48.3%) TT 25 (69.4%)	CC 4 (16.7%) TC 31 (51.7%) TT 11 (30.6%)	0.006*
Pneumocystis Carinii Pneumonia Infection	rs7439366	CC 53 (96.4%) CT 39 (84.8%) TT 19 (100.0%)	CC 2 (3.6%) CT 7 (15.2%) TT 0 (0.0%)	0.036*
Anemia	rs4149056	TT 75 (82.4%) CT 19 (86.4%) CC 3 (42.9%)	TT 16 (17.6%) CT 3 (13.6%) CC 4 (57.1%)	0.029*

Note: *p<0.05.

OATP1B1 among CC patients. The decreased MPA in hepatocytes in CC patients subsequently results in decreased OATP1B1 transportation activity in vitro, thus increasing plasma MPA levels.

This study found no differences in the MPA C_0/d among other SNP genotypes. However, a study indicated that UGT1A9-1818T>C was associated with a low dose-adjusted MPAG AUC_{0-12} h in Chinese renal transplant patients.[19] This is contrary to these research results and requires further investigation. One of the potential contributing factors for the contrary result was thought to be related to the variation in disease types.

For MMF-related adverse reactions, SLCO1B1 521T>C, UGT2B7 802C>T, and IMPDH1 −106G>A gene polymorphisms were associated with infections and adverse reactions of the hematologic system. The results of this study did not suggest that SNPs were associated with gastrointestinal side effects.

This study demonstrates a strong relationship between MMF-induced anemia and SLCO1B1 521T>C. Although no other available studies confirmed the same result, some studies suggest that SLCO1B1 521T>C is correlated with the risk of leukopenia.[27] Bouamar et al found that SLCO1B1 521T>C was not associated with the incidence of diarrhea or leukopenia in MMF-treated renal transplant recipients, which is consistent with the results of this study.

OATP1B1 mediates the transport of MPA from blood to hepatocytes, thus reducing the plasma MPA concentration. Michelon[28] et al found that the OATP1B1T>C gene polymorphism affected the transportation of MPA, increasing the plasma MPA concentration and thus affecting MMF-related adverse reactions. The proportion of MPA-related ADRs was significantly higher in patients carrying the SLCO1B1 521T allele than in C allele carriers. The SLCO1B1 521C variant allele was found to significantly reduce the probability of MPA-related ADRs. While this study suggested that SLCO1B1521T>C was associated with anemia, Michelon[28] et al found that the haplotype tagged by the SLCO1B1 521C allele was associated with a 75% risk reduction of MPA-induced adverse effects. The distribution of the CC type in the anemia group was much higher than that of the TT type. This may be due to the increased transportation of MPA by OATP1B1 before metabolism in TT type patients, resulting in increased MPA in hepatocytes and decreased plasma MPA concentrations. Thus, the incidence of adverse reactions was much lower. The results of this study showed that anemia is not affected by the SLCO1B3 334T>G gene polymorphism, which is consistent with the results of the larger sample reported in Jacobson et al's study.[29]

At present, most studies suggest that SLCO1B1 521T> C, SLCO1B3 334T> G and UGT2B7 802C>T are not related to gastrointestinal side effects.[26,28,30,31] The above conclusions are consistent with our study. Many studies have indicated that the gastrointestinal side effects of MMF are not related to the plasma level of MPA and that local exposure to MPA in the intestinal epithelium may be the cause of gastrointestinal side effects.[32] Khan et al[33] identified Midkine as a modulator of tight junction (TJ) permeability in MPA-treated Caco-2 monolayers, which contributed to the mechanism of MMF-related gastrointestinal side effects. However, other studies have come to the opposite conclusion[34] that it may be related to the differences in the types and identification of MMF-related gastrointestinal side effects. The definition of MMF-related gastrointestinal side effects should be further clarified. Moreover, the influence of UGT2B7 802C>t, UGT1A9 −1818T>C, and SLCO1B3 T334G on gastrointestinal side effects requires further investigation.

The results of this study suggested that IMPDH1-106G>A is correlated with MMF-induced infection. However, according to Ohmann,[35] the IMPDH1 −106G>A variant was associated with more serious gastrointestinal side effects. Ohmann et al[36] also found that MMF-related gastrointestinal side effects were associated with the IMPDH1 haplotype (containing IMPDH1 −106G>A) in pediatric heart transplant patients. It is hypothesized that IMPDH haplotypes are associated with individual differences in MMF-related adverse reactions.

IMPDH is the target enzyme of MPA. High IMPDH activity is related to poor efficacy. Patients with low IMPDH activity need a lower dosage to avoid adverse reactions.[37] Research has found that IMPDH gene polymorphisms can affect the occurrence of adverse reactions.[35,38,39] Our results showed that the IMPDH1 −106G>A gene polymorphism was significantly associated with infections. The distribution of the TC type in the infected group was significantly higher than that of the CC type (51.7% vs 16.7%) and the TT type (51.7% vs 30.6%), which is thought to be related to the lower IMPDH activity of TC type patients compared to those of CC type and TT type patients. However, some studies have come to the opposite conclusion. Michelon et al[28] found that IMPDH1 −106G>A was not related to the adverse reactions of MPA.

One study in organ transplant patients found that IMPDH1 −106G>A was associated with the risk of leukopenia in the first year after transplantation.[38] Kagaya[40] reached the same conclusion. Jacobson et al[29] believed that hematological toxicity, such as anemia and leukopenia, was not related to SLCO1B3 gene polymorphism. In this study, IMPDH1-106G>A did not affect leukopenia, which may be due to the influence of AcMPAG and the gene polymorphism affecting AcMPAG metabolism. The exact correlation requires further investigation.

Meanwhile, subgroup analysis was conducted based on the different pathogenic microorganisms. The results suggested that UGT2B7 802C>T is correlated with Pneumocystis carinii pneumonia infection. UGT2B7 is the main enzyme involved in the formation of AcMPAG, which is believed to be directly related to the adverse reactions of MPA.[13,41] Djebli[42] found that the 802C>T polymorphism had a significant effect on the production of AcMPAG in vitro. In this study, the UGT2B7 802C>T gene polymorphism was significantly associated with Pneumocystis carinii pneumonia. However, Pazik et al[31] found that the UGT2B7 802C>T allele was not related to infection. The types of infection were not investigated in this study, which may limit the study results.

At present, according to the limited information available, no previous studies have focused on SNP and MPA levels or adverse reactions in Chinese autoimmune disease patients. The results of this study indicated a significant association between SLCO1B1 521T>C and MPA C_0/d in Chinese autoimmune patients. It is suggested that SLCO1B1 521T>C affects MMF-induced anemia, IMPDH1 −106G>A is correlated with MMF-induced infection, and UGT2B7 802C>T is correlated with Pneumocystis carinii pneumonia infection. This study demonstrated the practicality of utilizing these specific SNPs for individualizing MMF dosing. However, some SNPs remain controversial and require further study. This study is limited by its relatively small number of patient samples; a larger sample size is needed to yield stronger research results. In addition, concentration has been described to be a poor indicator of MPA exposure, which is a limitation in this study. MPA concentration is the parameter that is routinely used in the majority of hospitals; therefore, determining the factors involved in the trough concentration is an important research direction.

Abbreviations

MMF, mycophenolate mofetil; MPA, mycophenolic acid; IMPDH, inosine-5′-monophosphate dehydrogenase; ADRs, adverse drug reactions; OATP, organic anion transporting polypeptide; MPAG, 7-o-glucoside (mycophenolic acid glucuronide); EMIT, enzyme multiplied immunoassay technique; PCRs, Polymerase chain reactions; AcMPAG, acyl-glucuronide (mycophenolic acid acyl-glucuronide); UGT, uridine 5′-diphospho-glucuronosyltransferase; SNPs, single-nucleotide polymorphisms; MPA C_0, steady-state plasma concentration of MPA; HWE, Hardy Weinberg equilibrium.

Disclosure

Professor Weihong Ge reports grants from Jiangsu Medical Innovation Team (Leading Talents) Project (Project Number:CXTDA2017041), outside the submitted work. The authors declared no other conflicts of interest. Qing Shu and Qingqing Fan are co-first authors.

References

1. Kawazoe M, Kaneko K, Yamada Z, et al. Efficacy of mycophenolate mofetil in Japanese patients with systemic lupus erythematosus. *Clin Rheumatol*. 2019:1–8.
2. Fialho SCMS, Bergamaschi S, Neves FS, et al. Mycophenolate mofetil in primary Sjögren's syndrome: a treatment option for agranulocytosis. *Rev Bras Reumatol*. 2012;52(2):297–299. doi:10.1590/S0482-50042012000200013
3. Jones RB, Hiemstra TF, Ballarin J, et al. Mycophenolate mofetil versus cyclophosphamide for remission induction in ANCA-associated vasculitis: a randomised, non-inferiority trial. *Ann Rheum Dis*. 2019;78(3):399–405. doi:10.1136/annrheumdis-2018-214245
4. Fakih R, Matiello M, Chitnis T, et al. Efficacy and safety of mycophenolate mofetil in progressive multiple sclerosis patients. *J Neurol*. 2018;265(11):2688–2694. doi:10.1007/s00415-018-9050-1
5. Shaw LM, Korecka M, Venkataramanan R, et al. Mycophenolic acid pharmacodynamics and pharmacokinetics provide a basis for rational monitoring strategies. *Am J Transpl*. 2003;3(5):534–542. doi:10.1034/j.1600-6143.2003.00079.x
6. Sherwin CMT, Sagcal-Gironella ACP, Fukuda T, et al. Development of population PK model with enterohepatic circulation for mycophenolic acid in patients with childhood-onset systemic lupus erythematosus. *Br J Clin Pharmacol*. 2012;73(5):727–740. doi:10.1111/j.1365-2125.2011.04140.x
7. Bullingham RES, Nicholls AJ, Kamm BR. Clinical pharmacokinetics of mycophenolate mofetil. *Clin Pharmacokinet*. 1998;34(6):429–455. doi:10.2165/00003088-199834060-00002
8. Zahr N, Arnaud L, Marquet P, et al. Mycophenolic acid area under the curve correlates with disease activity in lupus patients treated with mycophenolate mofetil. *Arthritis Rheum*. 2010;62(7):2047–2054.
9. Riskalla M, Somers E, Fatica R, et al. Tolerability of mycophenolate mofetil in patients with systemic lupus erythematosus. *J Rheumatol*. 2003;30(7):1508–1512.
10. Mok CC. Mycophenolate mofetil for lupus nephritis: an update. *Expert Rev Clin Immunol*. 2015;11(12):1353–1364. doi:10.1586/1744666X.2015.1087314
11. Ling J, Shi J, Jiang Q, et al. Population pharmacokinetics of mycophenolic acid and its main glucuronide metabolite: a comparison between healthy Chinese and Caucasian subjects receiving mycophenolate mofetil. *Eur J Clin Pharmacol*. 2015;71(1):95–106. doi:10.1007/s00228-014-1771-1

12. Li P, Shuker N, Hesselink DA, et al. Do Asian renal transplant patients need another mycophenolate mofetil dose compared with Caucasian or African American patients? *Transplant Int*. 2014;27(10):994–1004. doi:10.1111/tri.12382
13. Shipkova M, Wieland E, Schütz E, et al. The acyl glucuronide metabolite of mycophenolic acid inhibits the proliferation of human mononuclear leukocytes. *Transplant Proc*. 2001;33(1–2):1080–1081. doi:10.1016/S0041-1345(00)02424-6
14. Bernard O, Tojcic J, Journault K, Perusse L, Guillemette C. Influence of nonsynonymous polymorphisms of UGT1A8 and UGT2B7 metabolizing enzymes on the formation of phenolic and acyl glucuronides of mycophenolic acid. *Drug Metab Dispos*. 2006;34(9):1539–1545. doi:10.1124/dmd.106.010553
15. Shipkova M, Armstrong VW, Weber L, et al.; German Study Group on Mycophenolate Mofetil Therapy in Pediatric Renal Transplant Recipients. Pharmacokinetics and protein adduct formation of the pharmacologically active acyl glucuronide metabolite of mycophenolic acid in pediatric renal transplant recipients. *Ther Drug Monit*. 2002;24(3):390–399. doi:10.1097/00007691-200206000-00011
16. Bernard O, Guillemette C. The main role of UGT1A9 in the hepatic metabolism of mycophenolic acid and the effects of naturally occurring variants. *Drug Metab Dispos*. 2004;32:775–778. doi:10.1124/dmd.32.8.775
17. Guo D, Pang LF, Han Y, et al. Polymorphisms of UGT1A9 and UGT2B7 influence the pharmacokinetics of mycophenolic acid after a single oral dose in healthy Chinese volunteers. *Eur J Clin Pharmacol*. 2013;69(4):843–849. doi:10.1007/s00228-012-1409-0
18. Zhang WX, Chen B, Jin Z, et al. Influence of uridine diphosphate (UDP)-glucuronosyltransferases and ABCC2 genetic polymorphisms on the pharmacokinetics of mycophenolic acid and its metabolites in Chinese renal transplant recipients. *Xenobiotica*. 2008;38(11):1422–1436. doi:10.1080/00498250802488585
19. Xie XC, Li J, Wang HY, et al. Associations of UDP-glucuronosyltransferases polymorphisms with mycophenolate mofetil pharmacokinetics in Chinese renal transplant patients. *Acta Pharmacol Sin*. 2015;36(5):644–650. doi:10.1038/aps.2015.7
20. Shipkova M, Armstrong VW, Oellerich M, et al. Acyl glucuronide drug metabolites: toxicological and analytical implications. *Ther Drug Monit*. 2003;25(1):1–16. doi:10.1097/00007691-200302000-00001
21. Batko B, Krawiec P, Osieleniec J, et al. Mycophenolate mofetil in the treatment of selected connective tissue diseases. *Przegl Lek*. 2013;70(9):724–729.
22. Ruiz J, Herrero MJ, Boso V, et al. Impact of single nucleotide polymorphisms (SNPs) on immunosuppressive therapy in lung transplantation. *Int J Mol Sci*. 2015;16(9):20168–20182. doi:10.3390/ijms160920168
23. Bouamar R, Hesselink DA, Van Schaik RH, et al. Mycophenolic acid-related diarrhea is not associated with polymorphisms in SLCO1B nor with ABCB1 in renal transplant recipients. *Pharmacogenet Genomics*. 2012;22(6):399–407. doi:10.1097/FPC.0b013e32834a8650
24. Miura M, Kagaya H, Satoh S, et al. Influence of drug transporters and UGT polymorphisms on pharmacokinetics of phenolic glucuronide metabolite of mycophenolic acid in Japanese renal transplant recipients. *Ther Drug Monit*. 2008;30(5):559–564. doi:10.1097/FTD.0b013e3181838063
25. Kagaya H, Niioka T, Saito M, et al. Effect of hepatic drug transporter polymorphisms on the pharmacokinetics of mycophenolic acid in patients with severe renal dysfunction before renal transplantation. *Xenobiotica*. 2017;47(10):916–922. doi:10.1080/00498254.2016.1235742
26. Miura M, Satoh S, Inoue K, et al. Influence of SLCO1B1, 1B3, 2B1 and ABCC2 genetic polymorphisms on mycophenolic acid pharmacokinetics in Japanese renal transplant recipients. *Eur J Clin Pharmacol*. 2007;63(12):1161–1169. doi:10.1007/s00228-007-0380-7
27. Varnell CD, Fukuda T, Kirby CL, et al. Mycophenolate mofetil-related leukopenia in children and young adults following kidney transplantation: influence of genes and drugs. *Pediatr Transplant*. 2017;21(7):e13033. doi:10.1111/petr.13033
28. Michelon H, König J, Durrbach A, et al. SLCO1B1 genetic polymorphism influences mycophenolic acid tolerance in renal transplant recipients. *Pharmacogenomics*. 2010;11(12):1703–1713. doi:10.2217/pgs.10.132
29. Jacobson PA, Schladt D, Oetting WS, et al. Genetic determinants of mycophenolate related anemia and leukopenia following transplantation. *Transplantation*. 2011;91(3):309–316. doi:10.1097/TP.0b013e318200e971
30. Woillard JB, Picard N, Thierry A, et al. Associations between polymorphisms in target, metabolism, or transport proteins of mycophenolate sodium and therapeutic or adverse effects in kidney transplant patients. *Pharmacogenet Genomics*. 2014;24(5):256. doi:10.1097/FPC.0000000000000045
31. Pazik J, Ołdak M, Lewandowski Z, et al. Uridine diphosphate glucuronosyltransferase 2B7 variant p. His268Tyr as a predictor of kidney allograft early acute rejection. *Transplant Proc*. 2013;45(4):1516–1519. Elsevier. doi:10.1016/j.transproceed.2013.01.010
32. Bolin JP, Gohh R, Kandaswamy R, et al. Mycophenolic acid in kidney transplant patients with diabetes mellitus: does the formulation matter? *Transplant Rev*. 2011;25(3):117–123. doi:10.1016/j.trre.2010.12.003
33. Khan N, Binder L, Pantakani DV, et al. MPA modulates tight junctions' permeability via Midkine/PI3K pathway in Caco-2 cells: a possible mechanism of leak-flux diarrhea in organ transplanted patients. *Front Physiol*. 2017:438. doi:10.3389/fphys.2017.00438
34. Yang JW, Lee PH, Hutchinson IV, et al. Genetic polymorphisms of MRP2 and UGT2B7 and gastrointestinal symptoms in renal transplant recipients taking mycophenolic acid. *Ther Drug Monit*. 2009;31(5):542–548. doi:10.1097/FTD.0b013e3181b1dd5e
35. Ohmann EL, Burckart GJ, Brooks MM, et al. Genetic polymorphisms influence mycophenolate mofetil–related adverse events in pediatric heart transplant patients. *J Heart Lung Transplant*. 2010;29(5):509–516. doi:10.1016/j.healun.2009.11.602
36. Ohmann EL, Burckart GJ, Chen Y, et al. Inosine 5′-monophosphate dehydrogenase 1 haplotypes and association with mycophenolate mofetil gastrointestinal intolerance in pediatric heart transplant patients. *Pediatr Transplant*. 2010;14(7):891–895. doi:10.1111/j.1399-3046.2010.01367.x
37. Glander P, Hambach P, Braun KP, et al. Pre-transplant inosine monophosphate dehydrogenase activity is associated with clinical outcome after renal transplantation. *Am J Transpl*. 2004;4(12):2045–2051. doi:10.1111/j.1600-6143.2004.00617.x
38. Gensburger O, Van Schaik RHN, Picard N, et al. Polymorphisms in type I and II inosine monophosphate dehydrogenase genes and association with clinical outcome in patients on mycophenolate mofetil. *Pharmacogenet Genomics*. 2010;20(9):537. doi:10.1097/FPC.0b013e32833d8cf5
39. Cao W, Xiao H, Lai X, et al. Genetic variations in the mycophenolate mofetil target enzyme are associated with acute GVHD risk after related and unrelated hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2012;18(2):273–279. doi:10.1016/j.bbmt.2011.06.014
40. Kagaya H, Miura M, Saito M, et al. Correlation of IMPDH1 gene polymorphisms with subclinical acute rejection and mycophenolic acid exposure parameters on day 28 after renal transplantation. *Basic Clin Pharmacol Toxicol*. 2010;107(2):631–636. doi:10.1111/j.1742-7843.2010.00542.x
41. Gensburger O, Picard N, Marquet P. Effect of mycophenolate acyl-glucuronide on human recombinant type 2 inosine monophosphate dehydrogenase. *Clin Chem*. 2009;55(5):986–993. doi:10.1373/clinchem.2008.113936
42. Djebli N, Picard N, Rerolle JP, et al. Influence of the UGT2B7 promoter region and exon 2 polymorphisms and comedications on Acyl-MPAG production in vitro and in adult renal transplant patients. *Pharmacogenet Genomics*. 2007;17(5):321–330. doi:10.1097/FPC.0b013e32801430f8

Pharmacogenomics and Personalized Medicine

Dovepress

Publish your work in this journal

Pharmacogenomics and Personalized Medicine is an international, peer-reviewed, open access journal characterizing the influence of genotype on pharmacology leading to the development of personalized treatment programs and individualized drug selection for improved safety, efficacy and sustainability. This journal is indexed on the American Chemical Society's Chemical Abstracts Service (CAS). The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/pharmacogenomics-and-personalized-medicine-journal